Temperature experiments show a two-step unfolding of the protein. Above 45°C we have molten globule kind of loosened structure, and the complete unfolding occurs above 70°C. The unfolding is accompanied with aggregation of the protein. The aggregation can however be prevented by relatively low pressure of 0.2 GPa.
Using the above phase transition points the T-p phase diagram of parvalbumin was determined in presence of Ca^{2+}. It shows a very complex pattern, containing two different molten globule phases besides the native, unfolded and aggregated ones.

274-Pos Board B60
Protein Conformational Intermediates Characterized with Novel High Pressure EPR and CD Techniques
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Regulation of protein function is often linked to conformational intermediates that exist in equilibrium with the ground state. In many cases, these intermediate states exist as only a small fraction of the total protein conformational ensemble. It has been shown that high hydrostatic pressure is an invaluable tool for populating low-lying, excited states to levels amenable to spectroscopic detection. Here we demonstrate the usefulness of high pressure for populating such states as monitored by two novel techniques: High pressure electron paramagnetic resonance spectroscopy (EPR) and high pressure circular dichroism (CD). High pressure EPR was used in conjunction with site-directed spin labeling to monitor changes in local protein structure with applied pressure (up to 400 MPa). The relative partial molar volume and isothermal compressibility of each conformational substate was determined from the pressure dependence of the equilibrium constant determined from the continuous wave EPR spectra. High pressure CD was employed to detect global changes in secondary and tertiary structure at elevated pressure (up to 200 MPa). The combination of site-specific and global information provided by these techniques provides a more complete description of pressure excited intermediate states. Data from apomyoglobin and a cavity containing mutant (L99A) of T4 lysozyme are presented.

275-Pos Board B61
Volumetric Characterization of Interactions of Glycine Betaine with Protein Groups
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We report the partial molar volumes and adiabatic compressibilities of *N*-acetyl amino acid amides and oligoglycines at glycine betaine (GB) concentrations ranging from 0 to 4 M. We use these results to evaluate the volumetric contributions of amino acid side chains and the glycyl unit (–CH_2CONH–) as a function of GB concentration. We analyze the resulting GB dependences within the framework of a statistical thermodynamic model and evaluate the equilibrium constant for the reaction in which a GB molecule binds each of the functionalities under study replacing four water molecules. We calculate the free energy of the transfer of functional groups from water to concentrated GB solutions, ΔG_{tr}, as the sum of a change in the free energy of cavity formation, $\Delta\Delta G_C$, and the differential free energy of solute-solvent interactions, $\Delta\Delta G_I$, in a concentrated GB solution and water. Our results suggest that the transfer free energy, ΔG_{tr}, results from a fine balance between the large $\Delta\Delta G_C$ and $\Delta\Delta G_I$ contributions. The range of the magnitudes and the shape of the GB dependence of ΔG_{tr} depend on the identity of a specific solute group. The interplay between $\Delta\Delta G_C$ and $\Delta\Delta G_I$ results in pronounced maxima in the GB dependences of ΔG_{tr} for the Val, Leu, Ile, Trp, Tyr, and Gln side chains as well as the glycyl unit. This observation is in qualitative agreement with the experimental maxima in the T_M-versus-GB concentration plots reported for ribonuclease A and lysozyme.

276-Pos Board B62
Protein Stability and Macromolecular Crowding
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The environment inside cells is vastly different from the dilute, idealized conditions used in nearly all biophysical studies. Cells are exceptionally complex and contain macromolecules at concentrations exceeding 300 g/L. There are at least two profound consequences of the cellular environment that impact globular proteins. First, cells are highly ''crowded''. The resulting decrease in available volume should increase protein's stability. Second, because the bulk concentration of protein functional groups in cells is so high, nonspecific interactions between a protein and other cellular components, even if weak individually, can sum to large net, typically destabilizing, effects. in other words, crowding is a battle between the excluded volume effects and nonspecific interactions. We aim to identify the winner in different crowding environments, starting with inert synthetic polymers, moving to homogeneous protein crowders and ultimately to the endogenous cellular components of bacteria. We have already shown that excluded volume plays a predominant role for synthetic polymers, but nonspecific interactions with the test protein are stronger for more biologically relevant crowders.

277-Pos Board B63
Direct Quantification of the Effects of Osmolytes on Protein Unfolding and Refolding Pathways using AFM Techniques
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There is considerable interest in developing new effective therapies based on small molecules for the treatment of devastating pathologies, such as Alzheimer, Huntington and Parkinson diseases. Osmolytes are a class of small organic molecules found in all taxa that can profoundly affect protein stability and aggregation. Osmolytes such as sorbitol or trimethylamine-N- oxide can act as ''chemical chaperones'' by increasing the stability of native proteins, assisting refolding of unfolded polypeptides, inhibiting protein aggregation. However, the mechanism of protecting osmolytes action on protein folding and stability remains controversial, where the protein backbone seems to play a key role. Here we used single-molecule AFM to systematically analyze the effect of several naturally occurring osmolytes (sorbitol, sarcosine, TMAO, inositol, trehalose, proline, glycerol, and taurine) on the stability and folding kinetics on PKD domains which are normally exposed to high urea concentrations in the kidney. Our approach enables us to directly quantify the effects of osmolytes on the folded state and on the exposed protein backbone in the absence of chemical denaturants. Here we show that a mixture of different protecting osmolytes (e.g. 0.5M sorbitol + 0.5M sarcosine) counteracts the effects of 1M urea equally well as 1M sorbitol on the unfolding/refolding rates. These results indicate an additive effect of these protecting osmolytes on domain stability. However, we also found osmolytes mixtures that work synergistically on protein stability. Our results demonstrate a robust approach to study the mechanism of action of osmolytes at the single protein level.

278-Pos Board B64
Developing Solutes as Probes of Protein and DNA Processes
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Solutes have a broad range of effects on biopolymer processes, forming a spectrum from destabilizers like urea to more stabilizing osmolytes like proline, glycine betaine (GB) and KGlutamate to secondary structure inducers like trifluorethanol. To explain these effects and develop these solutes as probes of interface formation and large scale conformational changes in protein and DNA processes, we quantify the thermodynamics of their competition with water to interact with different types of biopolymer surface (e.g. aliphatic and aromatic C, polar and charged O and N) using model compounds displaying one or more surface type. Preferential interactions between the solutes and model compounds relative to their interactions with water are determined by osmometry or solubility and dissected using a novel coarse-grained analysis to obtain interaction potentials quantifying the solute's interaction with each significant type of biopolymer surface. Microscopic local-bulk partition coefficients *Kp* for the accumulation or exclusion of the solute in the water of hydration of these surfaces relative to bulk water are obtained. We used model compounds representing protein surface types to obtain *Kp* values for urea and GB, revealing that urea accumulates moderately at amide O and weakly at aliphatic C, while GB is excluded from both. These results provide both thermodynamic and molecular explanations for the opposite effects of urea and GB on protein stability, as well as deductions about strengths of amide NH - amide O and amide NH - amide N hydrogen bonds relative to hydrogen bonds to water. Urea and GB *m*-values for protein folding and other protein processes are interpreted and predicted using these interaction potentials or *Kp* values. We also determine interactions of these solutes with nucleic acid surface types to develop the ability to probe protein-nucleic acid interactions.
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279-Pos Board B65
Protein Hydration and Excluded Volume Interactions in Protein Folding and Stability: On the Mechanism of Protein Stabilization by Osmolytes
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Several osmolytes ranging in size from ~90-1600 cm3.mol-1 are shown to stabilize the folded states of apo-Mb at pH denaturing conditions. The action of